Goldberg, W.D.

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AN INVESTIGATION INTO ALBERTA GROWN ALKALOIDAL PLANTS

W. D. Goldberg

Department of Pharmacy

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W. D. Goldberg

I. INTRODUCTION

George P. Koch (14) in his paper "The Cultivation of medicinal Plants" stated -

> "War, with all its horrors, and terrible as the results may be, does produce some good. It stimulates production, compels efficiency, and teaches us to be more self-reliant."

This statement may well be taken as a reason for the marked increase, as he ably pointed out, in the cultivation of various medicinal plants in the United States immediately following 1914, as a result of being cut off from sources of supply.

This investigation limited itself to the growth and examination of some of the more widely used plants of the solanaceous group when grown under Alberta climatic and soil conditions.

Much work has been done, elsewhere, on this group of plants, particularly Atropa Belladonna, in regard to ideal growing conditions, locale, methods of cultivation, and the use of various fertilizers. The use of various

methods of extractions and determinations, in efforts to obtain the greatest possible alkaloidal yield, have also been reported.

II. LITERATURE REVIEW

a. Atropa Belladonna

Belladonna leaf consists of the leaves and tops of Atropa Belladonna, Linn, collected when the plant is in flower, and dried.

Belladonna, or Deadly Nightshade, is a herbaceous perennial with a fleshy, creeping root, from which arise several erect, branching stems, to a height of about three feet. The leaves occur usually in pairs and are usually of unequal size, oval, pointed, of a dusky green on the upper surface and paler beneath. The flowers are large bell-shaped, pendant, and of a purplish color. The fruit is a rounded berry.

The plant is a native of Central and South Europe where it is found growing in shady places, along walls and amidst rubbish. It grows vigorously, however, under cultivation.

During the past few years, much interest has been shown in the cultivation of this plant, particularly in the United States. Results show that a high grade of Belladonna

can be grown there (Berneman (1), Karr (10,11), Koch (13), Sievers (24).

Plants cultivated in California were found very rich in active constituents (U.S. Dispensatory XXI)*. The yield per acre of stems and leaves was somewhat less than one ton. The California experiment also showed that the alkaloidal content of Belladonna stems was equal to that of the leaves (0.51-0.82%) (28).

Sievers (25) found that no relation existed between the appearance of the leaf and its percentage content of alkaloid. Specimens, however, he said, which are musty or contain too much stem, should be rejected as weak in alkaloidal content.

Koch (13) studied the germination of seeds of Atropa Belladonna in parts of the United States and found that the seeds germinate slowly. However, it was found by workers at the University of Minnesota that the treatment of seed with concentrated sulphuric acid for forty-five seconds and washing repeatedly with distilled water, would produce uniform germination in about fifteen days. Sievers (23), however, disputed this result.

Koch (13) points out that Atropa Belladonna germination is greatly enhanced by planting in a hothouse, and when the plant has grown to a height of about four and a half inches, over a period of about three months, it is set out into the experimental field.

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* Published by J. B. Lippincott Co., Philadelphia.

Karr (10), however, showed that the cultivated plant contained very little more alkaloid than did the wild. He stated that nitrogenous fertilizers tended to lower alkaloidal content because of larger leaf surface. This conclusion was reached by observations and determinations carried out on plants grown in England. Karr (11) also found that in whatever latitude Atropa Belladonna is grown, it was conclusive that the composition of the soil, the use of fertilizers and seasonal conditions made for very small variations. His plants were grown in a chalky soil.

As to moisture, not many workers indicate the needs of Atropa Belladonna. Koch (13) found that two-thirds as much weight of leaves and stems was harvested where moisture to the extent of one-half the physical optimum of the soil was applied, as was produced where the conditions of moisture were at the optimum.

Goris and Deluard (7) cultivated Atropa Belladonna in places both exposed to the sun and in the shade and found that the plants exposed to the sun produced three times as many leaves as those in the shade. The alkaloidal content, they reported, was also much higher. This finding agreed very well with the findings of Karr (10).

The B.P. directs that the leaves be gathered at flowering time and should not contain more than 20% of stems. But with, of course, always the resulting alkaloidal content in mind, Borneman (1) states that the plant can be

harvested any time in the fall and some time before the first sign of the leaves turning yellow, as at that time there is a rapid deterioration of alkaloids. Also regarding the amount of stem to be collected, most workers appear to agree that the stems may be used as well as the leaves and the alkaloidal content still not fall below the B.P. requirements.

All workers are agreed that the leaves, on being gathered, should be dried as rapidly as possible. This is well shown by Borneman (1) and others. Koch (13) states that the leaves should be rapidly dried at a temperature of 55^{o}-60^{o} as at this temperature, the dried leaves yielded a maximum of alkaloidal content.

b. Hyoscyamus niger

Hyoscyamus consists of the dried leaves and flowering tops of Hyoscyamus niger (Linn.) and contains not less than 0.05% of the alkaloids of Hyoscyamus, calculated as hyoscyamine.

There are about fifteen species of the genus Hyoscyamus known. They are found in the Canary Islands, Europe, Northern Africa and Asia. The plant is found in north and east sections of the United States, occupying waste plots, old gardens, cemeteries, etc. It is not a native of North America, but was introduced from Europe.

The plant occurs in two forms, annual and biennial. It has a long, tapering, soft, whitish root resembling parsley, for which it has been mistaken, with disastrous results.

In England and on the continent, Europe, it grows along the roads, around villages, in rubbish and in waste places. Both varieties were formerly cultivated in England, but now the biennial is chiefly grown.

Gieger and Hesse (1883) were the first to demonstrate the existence of an alkaloid in Hyoscyamus niger. Ladenburg (1880) made the statement that there were two alkaloids present, and these were subsequently shown to be crystallizable hyoscyamine, $C_{17}H_{23}O_3N$ and the other, amorphous hyoscine, $C_{17}H_{21}O_4N$.

Hyoscyamine is the dominant alkaloid though some hyoscine and atropine do occur.

Henry (9) finds the following total determination of Hyoscyamus niger:

Leaves	0.045%-0.08%
Roots	0.15 -0.17
Stems	0.06 -0.10
Tops	0.07 -0.10

J. A. Borneman (1), who carried out the cultivation of various solanaceous plants in Pennsylvania, stated that Hyoscyamus niger was the most difficult of all to grow. The specimens obtained were usually beautiful, but the assays were almost always disappointing and rarely gave an alkaloidal content in excess of 0.06%. His conclusion was

that unless it would be possible to bring the assay up to standard it would not pay to cultivate the plant.

Experiments by Newcomb and Haynes (19) showed that Hyoscyamus niger cultivated in Minnesota yielded very high percentages of total alkaloids, the range being from 0.096% to 0.1561%. They recommended (as they did for Atropa Belladonna) the treatment of the seed with concentrated sulphuric acid before planting, and they found that they obtained a fairly uniform germination in from twelve to fifteen days.

Klan (12) states that with the growth of the germinating plant, the quantity of alkaloid in its organs decreases. He states that the order of alkaloid content in Hyoscyamus niger (both annual and biennial) is

1. Root
2. Flowering tops
3. Fruits
4. Leaves
5. Stems

The plant growth is rapid and has a characteristic fetid odor, which disappears on the drying of the leaf. The growth is heavy and the leaves are large, though varying in size, the lower leaves being considerably larger than those above.

Patu (20) in his growth of the plant found that mildew had completely covered the radicle leaves of the first year Hyoscyamus, and found in these leaves a lowered alkaloidal content.

As, in referring to A. Belladonna, the writer finds that workers seem agreed that the stems of Hyoscyamus niger can be used as well as the leaves at harvest time, and the official requirements still met. This was the finding of Koch (15, 16).

c. Datura Stramonium

Stramonium consists of the dried leaves and flowering tops of Datura Stramonium (Linn.) and Datura tatula (Linn.).

Datura Stramonium (the thornapple) is an annual plant of rank and vigorous growth, usually about three feet high, but growing in exceptionally rich soil may attain a growth of six feet. The root is large, greyish-white, with numerous rootlets. The stem is round, erect, shiny and with numerous large spreading branches. The leaves are large, five or six inches in length, dark green on the upper surface with a lighter green on the lower surface. The flowers are large, solitary, with a funnel-like corolla. The fruit is a large, fleshy, ovoid, 4-valved capsule, thickly covered with very sharp spines and containing numerous flattened seeds, all attached to a central placenta. It opens at the summit at ripening, and on drying the seeds are easily removed.

It is doubtful to what country Datura Stramonium first belonged. European botanists have referred it to North America, while American botanists return it to Europe.

Some consider it as having originated in South America or Asia, and it is probable that its original habitat is to be found somewhere in the east.

It is reported to grow wild and abundantly in Southern Russia, from the borders of the Black Sea east to Siberia. In the United States, where it has probably come by seed on board ship in dirt ballast, it is found in waste spots, road sides and rank soil. Where the plant grows abundantly, it may be recognized by its rank odor. But notwithstanding its wide growth in the wild state, it is being cultivated in order to obtain a drug of uniform quality.

The American supply of the drug comes mainly from Europe, chiefly Holland, England and Germany.

Koch (14) in his paper "The Cultivation of Medicinal Plants" states that Stramonium is a weed in nearly all parts of the United States, and as a weed it attains considerable growth but is always better under cultivation. Ordinarily, after seeding, he says, the plant requires no special care, other than occasional cultivation.

But Miller and Meader (18) found that cultivation, particularly on D. Stramonium and D. tatula, with and without fertilizer, had increased the alkaloidal output. This came as a contradiction to the findings of Karr on Belladonna (10) when he found that the greatest growth was in unusually dry and sunny seasons (0.68% alkaloids). Also in a patch with much sun and no fertilizer, he obtained 1.035% alkaloids.

All parts of the plant are active. The parts, according to most workers, may be gathered at any time from the appearance of the flowers to the autumn frost. A.R.L. Dohme (3) examined Stramonium to determine the value in alkaloids of the various parts of the plant. He found that, in general, the fresh parts yielded more than the dried, and that the order of alkaloidal content in the organs was as follows: stems, seeds, leaves, root.

Gieger and Hesse (1833) first isolated an alkaloid which they called daturine, and which later was found to be identical with hyoscyamine. There is also present in the drug traces of scopolamine. George P. Koch (13), during his various researches on the solanaceous plants in regard to their relative alkaloidal content, compared the relative values of the stem and leaves of Datura Stramonium. He states that the whole plant, with or without the root, can be used without fear that the total alkaloidal value will fall below B.P. or U.S.P.X. standards.

d. Datura Metel

The Datura leaves are the leaves of Datura Metel and Datura fastuosa which are annual plants occurring chiefly in India and attain a height of three to four feet. While the plant Datura Metel is indigenous to India, it has been carried to almost all tropical regions. The leaves are

slightly toothed or wavy-margined, and the flowers are trumpet-shaped and white. Except for the leaves, Datura Metel very closely resembles Datura fastuosa.

In India, the leaves are used as an equivalent to Belladonna and Stramonium.

Though not a great deal of work has been done on Datura Metel, it contains chiefly hyoscine (scopolamine) (9) with occasionally small amounts of hyoscyamine and atropine.

The plant, though belonging to the Solanaceous group of plant drugs, is not official, but forms the chief source of hyoscine.

The alkaloid is most commonly extracted from the leaves, which contain 0.25-0.55% of alkaloids, the other parts of the plant yielding varying amounts of alkaloids as follows:

Fruits	0.12%
Roots	0.1 - 0.22%
Seeds	0.23- 0.50% (28)

III. OBJECT OF INVESTIGATION

This investigation was prompted by the desire to know if the plants, normally grown in central and southern Europe and in southern England, could be successfully grown in our normal Alberta climate, with its comparatively short growing season, and to see if these plants would produce alkaloidal contents, at least equal to the B.P. requirements.

No added cultivation, other than periodic watering during the dry season and occasional weeding, would be used.

It was then with a sense of questioning if Alberta could produce at least some of its own medicinal plants successfully that this investigation was undertaken.

The work of Newcomb and Haynes (19) mentioned previously, in connection with solanaceous plants in Minnesota, acted as an incentive for the investigation, since the growing conditions of Minnesota and Alberta are very similar.

IV. METHODS (A review)

Much work has been done on the extraction and assay of the solanaceous plants, particularly Atropa Belladonna.

Many methods have been evolved, each method giving the particular worker fairly consistent results, but the results of the various workers remaining out of agreement. This may be due to personal error or to manipulation, as various workers using the same method obtain varying results. Similarly Sievers (24) showed a variation of the alkaloidal content of the leaves of the same plant, ranging from 0.110% to 0.766%.

The chief point of contention in the actual method of assay of particularly the solanaceous plants has been the relative instability of the alkaloids contained

therein. Recent workers, among them Watkins and Palkin (26), Dekay (27) and Jordan (2), Evans and Goodrich (5), Evans and Davy (4) are satisfied that the alkaloids are stable enough to permit of extraction by a hot ether, or other solvent, apparatus. Schow and Bjerregaard (21), while working with the sterilization of various substances, found that solutions of atropine could be heated at 120° for twenty minutes without danger of decomposition. Dekay and Jordan (2) formed the conclusion, based on their experiments with the alkaloids, that the alkaloids are much more stable than they are usually assumed to be. When chloroform solutions of them are evaporated, they can be heated at water-bath temperature for one or two hours, without decomposition. Watkins and Palkin (26) found that the alkaloids heated in acid or alkali on a water-bath showed no apparent loss. Consequently, many new methods have been evolved for the extraction of solanaceous plants.

Before going on to the newer methods, established methods were first examined for their value, keeping always in mind the fact that the B.P. still considers these alkaloids too unstable in solution to be extracted by any means other than the maceration and cold extraction.

a. B.P. 1914 Method

This method directed the maceration of the powder in #60 powder, directly in the percolator, fitted with a

tap and plugged with cotton. Maceration was allowed for one hour, then percolation carried on slowly, into a separator containing N/1 sulphuric acid. The final chloroformic residue of alkaloids was evaporated and titrated with 10 mils of 0.05 N sulphuric acid and 0.05 N sodium hydroxide, using tincture of cochineal as indicator.

b. B.P. 1932 Methods

The new methods for A. Belladonna and D. Stramonium are identical.

The powder is directed to be of a #60 fineness and is macerated in a flask for one hour, in contact with ammonia, alcohol, and ether. It is transferred to a percolator plugged with cotton and percolated for not more than three hours or until total extraction of alkaloids is effected. To the percolate is added 0.5 N hydrochloric acid and the alkaloids shaken out in the usual manner. The final chloroform solution of alkaloids is evaporated to dryness and dried for half an hour at 100°. The residue is titrated using 0.02 N sulphuric acid and 0.02 N sodium hydroxide and methyl-red as indicator.

For Hyoscyamus, which contains a smaller amount of alkaloids, much the same procedure is used, but since forty grammes of drug are used, the standard percolator is used. Maceration and percolation are similar to those used for Belladonna, but percolation should not require more

than two hours. Since, however, the alkaloids of Hyoscyamus are more sensitive to heat than are the others, the mixed acid solutions of alkaloids are made alkaline and evaporated in vacuo at a temperature not exceeding 40°. It is then extracted with chloroform as usual, the chloroform evaporated off and the residue dried at 80° for two hours. The residue is titrated using 0.02 N sulphuric acid and 0.02 N sodium hydroxide, and methyl-red as indicator.

c. The Method of Rosenthaler

For the total extraction of the drug plants, the procedure as outlined by Rosenthaler in his "The Chemical Investigation of Plants" (1930)* appeared to be the most thorough, and is a method by which all constituents occurring in the plant may be isolated and recognized.

d. The Method of Watkins and Palkin (27)

This method was devised particularly for the evaluation of Hyoscyamus. The alkaloidal yield by this method has shown results in some cases as much as three times as great as that obtained by the U.S.P. IX and X methods.

The essential steps in this mechanical extraction are:

i. Treatment of the mass of crude drug with an alkaline medium to liberate the alkaloid. Macerate over night.

* Published by G. Bell and Sons, Limited, London.

ii. Continuous extraction by means of the device described, using ether as a solvent.

iii. Purification process of the alkaloidal residue, during which chlorophyll, tars and other extractives are removed.

iv. Extraction of the alkaloids from aqueous solution by means of another continuous device.

v. Titration of the alkaloids.

The concentration of ammonia in the original maceration has been the subject of considerable enquiry, and it may be in this connection that the varying results occur with different workers.

It is conceivable that too low a concentration of ammonia will not completely free the alkaloids from the crude drug, while too great a concentration is not advisable because of the instability of the alkaloid hyoscyamine.

With this in mind, Watkins and Palkin extracted several samples with all conditions being uniform, but varied the concentration of ammonia.

They found that 7 cc. of 16 N ammonia proved the most efficient, as may be seen from the following table taken from their paper.

TABLE I

Effect of ammonia concentration on yield of alkaloid

Ammonia		Alkaloid	
Quantity cc.	Normality	Mgm.	Per cent
5	5	19.09	0.159
		16.49	0.138
4	16	20.54	0.171
		21.12	0.176
7	16	20.54	0.171
		20.54	0.171
9	16	20.54	0.171
		21.12	0.176

These workers used 0.02 N acid and 0.02 N alkali and used methyl red as an indicator.

e. The Method of DeKay and Jordan (2)

These workers, like Watkins and Palkin, used a mechanical extraction apparatus in much the same manner as did Watkins and Palkin, except that they used the regulation Soxhlet apparatus instead of one specially devised. The assay was essentially the same, except that these workers extracted completely the volatile bases and determined them.

They concluded, from their experiments, that the alkaloids of Hyoscyamus (the subject of their paper) are more stable than usually thought. They determined also, that most procedures extract certain volatile bases which they determined as trimethylamine and a primary amine, and the indication of the presence of dimethyl-amine, along with the alkaloids.

DeKay and Jordan used 0.02 N acid and 0.02 N alkali in their titration of the alkaloid, and methyl red as indicator.

f. Abstract of Proposed Changes to U.S.P.X. (29) and U.S.P.X. Method

This paper dealt with two new proposed methods for assaying crude drugs, i.e.,

1. Aliquot part method (by maceration only),

2. Total extraction method (by maceration and percolation).

The second procedure more closely resembles the B.P. method so it only will be considered.

The drug is placed in a small cylindrical percolator (see Plate I, Fig. 1) previously prepared by packing the outlet with cotton. Add solvent to completely saturate the drug, mix, cover the percolator, allow to stand five minutes and add the ammonia solution and mix again.

Percolator Types

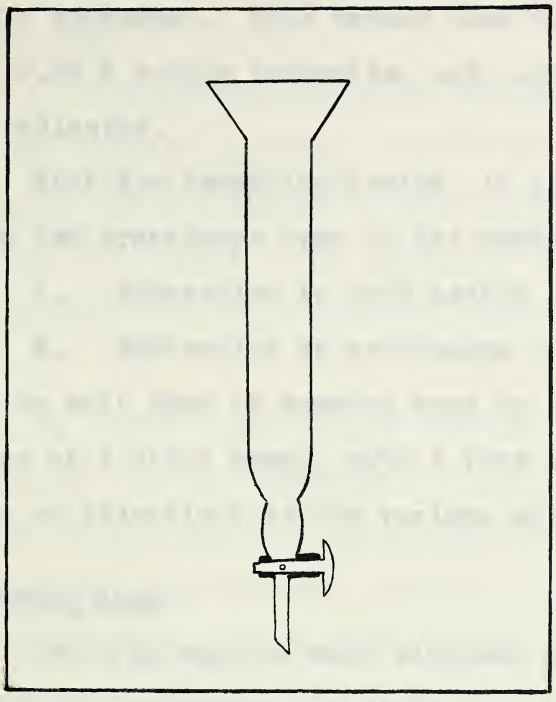

Fig. 1

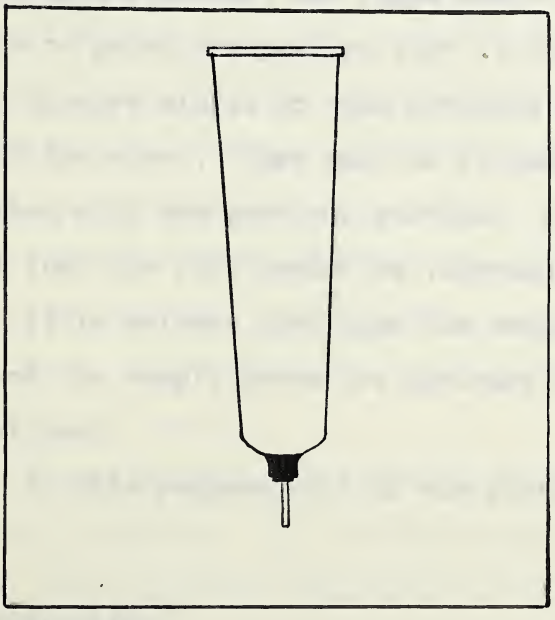

Fig. 2

Plate I

After macerating for one hour, percolate slowly until completely extracted. This method uses 0.10 N sulphuric acid and 0.02 N sodium hydroxide, and cochineal or methyl red, as indicator.

With the foregoing review, it appears then that there are two procedures open to the worker, viz.,

1. Extraction by cold method (B.P. and U.S.P.X.),
2. Extraction by continuous or hot method.

It might be well then to examine step by step the basic principles of a plant assay, with a view to determining the value or objections to the various methods outlined.

1. The crude drug.

The drug must be well divided, at least to a #60 powder and must represent the whole drug. It is insufficient to grind the powder, sift it through a #60 sieve and discard midrib or stem portions which refuse to go through the sieve. They must be further ground and then incorporated with the previous portion. It is further important that the fine powder be thoroughly mixed.

It is evident that upon the completeness and fineness of the sample rests the accuracy of the resulting determinations.

In this respect, all of the foregoing methods agree.

2. The extraction.

Upon the differences of opinion as to the relative stability of the solanaceous alkaloids rests the fact that

there are so many varied procedures advanced for the assay of a plant drug.

All procedures agree upon the maceration, the hot methods, however, directing maceration over night, while the cold methods macerate for not more than two hours. With the proper concentration of ammonia, it is conceivable that two hours should be sufficient to free all alkaloids.

The tendency to the production of an emulsion when a solution containing vegetable extractives is shaken with an immiscible solvent, forms a great and common difficulty. This difficulty, however, is now almost wholly surmounted in most methods by the addition of alcohol in the first shaking out with acid, instead of acid alone. Emulsions in the hot method are not very common. All procedures extract considerable coloring and other matter in the original extractive, and this often interferes in the final determination by coming through in traces. The procedure by DeKay and Jordan (2) and Watkins and Palkin (27) precipitates this coloring matter by adding acid. The solution is then filtered and the chlorophyll left behind.

The alkaloidal residue is not treated similarly in the various procedures. All methods agree that there is extracted, along with the alkaloids, an appreciable amount of volatile principles and direct the heating of the final

residue at varying temperatures, governed by the relative stability of the alkaloid.

In this respect, independent workers agree that the solanaceous alkaloids are more stable than they are generally reported to be (vide p. 13). This thought is coming into the methods of the B.P. and U.S.P.X. since the residues are heated, in the case of Belladonna and Stramonium, at 100° for one-half an hour.

The method outlined by DeKay and Jordan isolates, weighs and calculates the actual percentage present of volatile principle, while other methods simply heat the residue to drive off these volatile principles and then titrate.

The indicators in all cases suggested are cochineal or methyl red and the use of one or the other is left to the discretion of the worker. Self (22) to a certain extent defends cochineal, though methyl red is preferred. To obtain high accuracy with either indicator, Self suggests that the volume of titration liquid be kept as low as possible, especially in assays where the weight of alkaloid obtained is very small. This expedient, he says, produces a much sharper end-point.

Evers (6) suggested that Brom-phenol-blue would prove the best indicator for titrating the solanaceous alkaloids, and that methyl red ranked next. The range of pH of Brom-phenol-blue is 2.8-4.6; the range of pH of methyl red is 4.2-6.2. Mellon and Tigelaar (17), however, found

that, though methyl red did not have quite the best pH range for titration purposes, yet the error involved in the titration of atropine by methyl red is so small it may be disregarded.

On looking back over the above review of the methods, one is struck with the apparent similarity in procedures in all steps, save that of extraction. Here the opinion and findings of the individual worker enter to make up his technique and thus we find, as mentioned above, one system extracting by the cold percolation process, and the other extracting by the hot mechanical method. Modern workers lean toward the latter procedure, insisting that the alkaloids of Solanaceae are not unstable.

Of the various methods described, the writer decided to choose two to be used in the assays, in a comparative manner. The methods, also, were to consist of both the cold percolation method and the hot, continuous method. With a view of choosing the two most promising methods, the above were considered in the order in which they appear.

(a) The B.P. 1914 method was used only on Stramonium. It was discarded in favor of the B.P. 1932 method for its advantages listed under (b).

(b) The B.P. 1932 Method. This method is outlined on page 14 and was used in a comparative sense for our plants and also as a check on various other methods.

The B.P. 1932 method has several distinct advantages and changes which tend toward greater accuracy over the B.P. 1914 method. An examination of the two methods

shows the following changes, with their advantages or disadvantages.

i. Maceration - The B.P. 1914 directs "Into a small stoppered glass percolator --- introduce 10 grammes of ....", while the B.P. 1932 directs "Introduce 10 grammes of powder into a flask". Both are then macerated for one hour, but the B.P. 1932 powder must, of course, be transferred to the percolator.

Maceration, by the two methods, it would seem would be more complete in the latter, since it is much more thorough to shake a flask than to shake a percolator, even if it could be tightly corked. A second advantage of the latter method is that no channelling can take place with resulting incomplete percolation.

ii. Emulsification - Emulsificiation in the assay has been fairly well overcome in the B.P. 1932 method by the addition of alcohol to the solvent. This, as pointed out by Self (22), prevents emulsification, which occurred commonly in the 1914 method.

iii. First acid extraction - At this point it would appear that the extraction of alkaloids is more complete since the ether-chloroform extract is run into the acid. In the 1932 method, the acid is added to the extract and shaken.

iv. Washing of acid solution - The B.P. 1932 directs that the acid solution containing the alkaloids be

washed with chloroform, the chloroform run through acid and then discarded, while this procedure is not carried out in the B.P. 1914 method. This is a distinct advantage since any coloring matter carried down from the original volatile solvent extract is removed.

v. Washing of chloroform extract - The B.P. 1914 collects the chloroformic extract and directly dries it, dissolves it in ether, dries and titrates it, while the B.P. 1932 directs that the chloroform extract be washed with water before being dried, alcohol added, and titrated . This point is commented upon, as mentioned, in the Pharmacy Journal (30), page 30.

In all determinations carried out in this investigation, the final alkaloidal solution was washed with water to eliminate any source of error in that direction.

vi. Titration - The B.P. 1914 directs the use of 0.05 N acid and 0.05 N alkali, while the B.P. 1932 directs the use of 0.02 N acid and 0.02 N alkali. This is an important step toward greater accuracy, and in some cases a sharper end point.

vii. Indicator - The indicator cochineal, official in 1914, is now replaced by methyl red, a much more accurate and efficient indicator, as shown in the discussion by Self (22), Evers (6), Mellon and Tigelaar (17) and others on page 22.

In all determinations by the B.P. 1932 process, on Belladonna and Stramonium, the percolator used was very similar to that described in the U.S.P.X. method (Plate I,

fig. 1). This percolator proved more convenient for small quantities of drug.

In the extraction of Hyoscyamus by the B.P. 1932 process, the method is essentially that of Belladonna, with modifications firstly for the large quantity of drug necessary (since there is only a small amount of alkaloid present) and secondly, the reported greater instability of the alkaloids.

This is taken care of, in the case of the large amount of drug, by using a larger percolator as shown in Plate I, fig. 2, and which is of the standard type. Since the quantity of drug is large, it is apparent that at the acid extraction of the alkaloids, the volume will be large. Before being extracted with chloroform, the mixed acid solutions are made alkaline and evaporated in vacuo to about 50 cc. at a temperature not exceeding 40°.

The alkaloids are shaken out with chloroform, washed with water, the chloroform removed and the alkaloids dried at 80° for two hours. This removes any volatile bases present and so makes the final titration more accurate. The alkaloids are titrated with 0.02 N sulphuric acid and 0.02 N sodium hydroxide, using methyl red as indicator.

In using the above method for Hyoscyamus, the greatest objection to it is the length of time required for completion. It is always advisable, where possible, to complete an assay on the same day as started, since

the alkaloids are of such a nature that standing over night may completely change the final result. But with the B.P. 1932 assay of Hyoscyamus, a complete assay is impossible in one day.

The B.P. 1932 processes were used as outlined in the text with the two following changes:

1. The liquid was percolated as directed, but instead of adding the 0.5 N hydrochloric acid to the percolate, the acid was placed in the separator prior to percolation with the immiscible solvent. This was done for two reasons:

(a) To avoid any possible leakage since the acid layer would remain at the bottom, and

(b) To insure complete conversion of the alkaloids to hydrochlorides.

2. All alkaloidal residues, on the addition of the specified 0.02 N acid were warmed on a water bath to insure complete solution. The flask was then cooled at room temperature and the titration carried out.

The results obtained by the B.P. 1932 methods proved quite consistent, and, as it is at present outlined, the method may well be said to be one of the most efficient of alkaloidal assays. Self (22), in his review of alkaloidal assays of the B.P. 1932, stated that out of a total of 46 alkaloidal assays in the B.P. 1932, 29 are quite new and only three have remained practically as they were. The changes, he says, are almost wholly of

British origin, and it may be claimed that in the assay section of the work the B.P. is still well in front. The aims, of course, have been to make the assays simpler and more accurate, but accuracy always remained the prime object.

(c) The method outlined by Rosenthaler (see "The Chemical Investigation of Plants" (1930)* was not used in this investigation as it is a method for total extraction.

(d) The Method of Watkins and Palkin (27). This method is essentially similar to that used by DeKay and Jordan, which will be described later. The methods, however, are different, in that Watkins and Palkin used a specially devised apparatus while DeKay and Jordan used a Soxhlet apparatus. Since the methods are alike, and similar results may therefore be expected, and since the DeKay and Jordan process provides certain advantages, the Watkins and Palkin method was omitted in its favor.

(e) The Method of DeKay and Jordan (2). This method was outlined by DeKay and Jordan after exhaustive experiments, both on pure alkaloids and on the crude drug.

The extraction consists of mechanical extraction by a Soxhlet apparatus, the drug having been macerated over night.

The procedure follows:

Samples I and II (20 grammes each) in 60 powder were placed in Soxhlet thimbles, dropped into place in the extractors and moistened with a mixture of 7 cc. strong solution of ammonia, 8 cc. of alcohol and 16 cc. of ether.

It was well mixed with a stirring rod and macerated over night. The following day, it was extracted with ether over a water bath. The extraction required, as a rule, three to four hours. The dark green extract, so obtained, was evaporated over a water-bath, to about 12 cc. and then 8 cc. of 0.1 N sulphuric acid and 8 cc. water added. The evaporation was continued until all the ether was removed. At this point, the coloring principle was precipitated. The liquid (acid solution of alkaloids) was filtered into a separator and the precipitated chlorophyll residue redissolved in chloroform, 0.1 N sulphuric acid again added, and again the chlorophyll was precipitated. The mixture was again evaporated free of chloroform and the liquid remaining again filtered into the separator through the same paper. The combined filtrate was made basic with dilute ammonia (using litmus) and the alkaloids extracted by shaking with successive portions of chloroform, until completely extracted, as shown by a test with Mayer's reagent.

The chloroform solution was evaporated to a low volume and the residue dried at water bath temperature for 15 minutes. A brownish aromatic residue remained. This residue was redissolved in chloroform, again evaporated and again dried for 15 minutes. This process was repeated for the third time.

At this point, the residue may be dissolved in about 10 cc. of chloroform and then 15 cc. 0.02 N sulphuric

acid added, the chloroform evaporated off and the titration carried out, using methyl red as indicator, or the residue may be treated for the removal of any volatile bases present. The latter was the process used in this investigation in most cases.

This process consisted of passing air through a sulphuric acid wash bottle and a $CaCl_2$-Na_2CO_3 tower and then through a $CaCl_2$ U-tube, heated at 40°. This frees the air of moisture and warms it. This warm dry air was then slowly passed over the alkaloidal residue and finally into the flask containing 0.1 N hydrochloric acid (see Plate II, figs. 1 and 2).

The volatile bases thus carried over by the air into the 0.1 N hydrochloric acid are there held. After one hour, the hydrochloric acid solution is heated to dryness and the residue weighed.

The alkaloidal residue is then titrated as described above.

This method appeared to be a thorough procedure for the extraction of alkaloids.

It provided a method for the hot extraction of alkaloids and would, indirectly, indicate if the alkaloids of the Solanaceae could be extracted by such a method without loss.

The weighing of the drug into the thimble, the maceration and extraction while in this receptacle without the disadvantage of transferring from a flask to a percolator, is obviously an advantage both from the views of expediency and accuracy.

The extraction of volatile bases (DeKay and Jordan)

A - H_2SO_4 drying bottle
B - $CaCl_2$-Na_2CO_3 tower
C - $CaCl_2$ U-tube (at 40°)
D - Alkaloidal residue (at 40°)
E - 0.10 N HCl
F - Trap containing H_2O
G - To pump

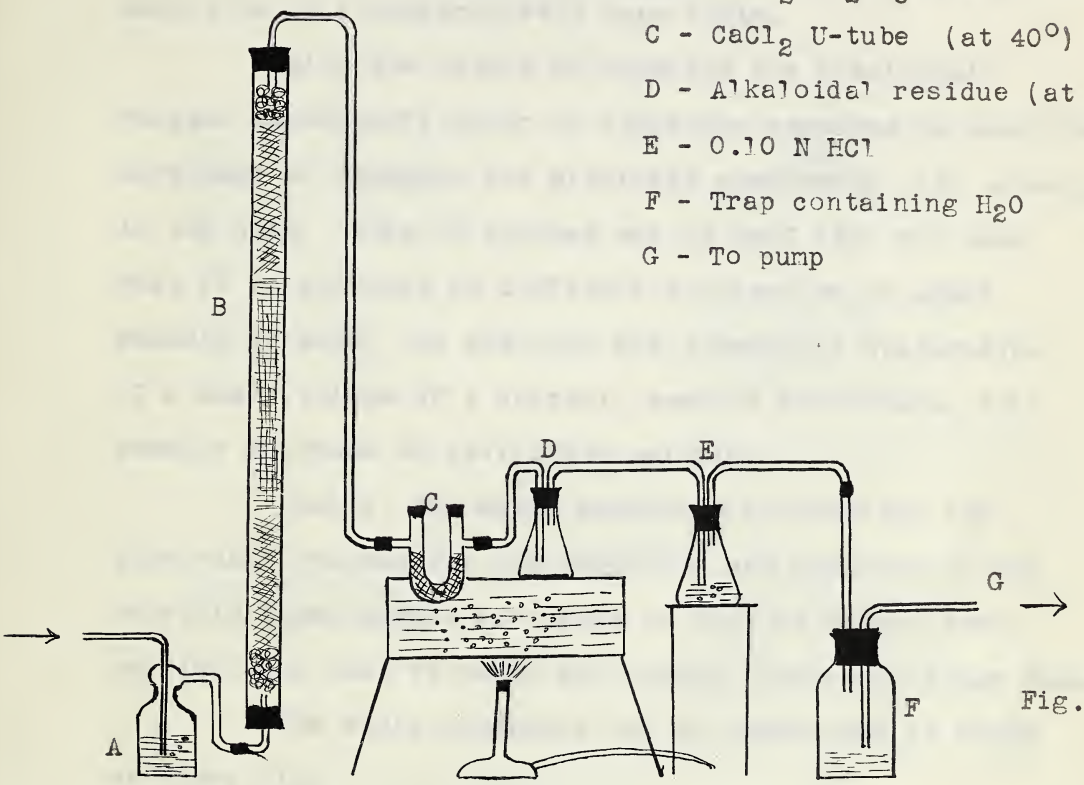

Fig. 1

Fig. 2

Plate II

On obtaining the ether extract, the precipitation of chlorophyll and other foreign extractives with acid seemed an important feature, as the alkaloidal acid solution should be in a comparatively pure state.

Also the method of handling the alkaloidal residue immediately prior to titration appeared to have the advantage of bringing the alkaloids completely into solution in the acid. This is pointed out by Self (22) who says that if an alkaloid is difficult to dissolve in small amounts of acid, the addition and subsequent evaporation of a small volume of a solvent, such as chloroform, will usually be found to facilitate solution.

Lastly, the means described of handling the alkaloidal residue for the isolation and weighing of the volatile bases seemed advisable in view of recent work, particularly that of DeKay and Jordan, regarding these bases.

The whole procedure may be summarized as shown on Plate III.

After a few determinations with this process, it was found advisable to make the following modifications:

(a) After the complete removal of the chloroform by evaporation, the flask containing the alkaloidal acid solution and the chlorophyll residue was cooled to room temperature whereby the precipitate was coagulated. Filtration was then carried out by decantation. It was found that if the solution was completely cooled after each evaporation, very little chlorophyll was carried over.

Plan of DeKay and Jordan process for alkaloidal assays

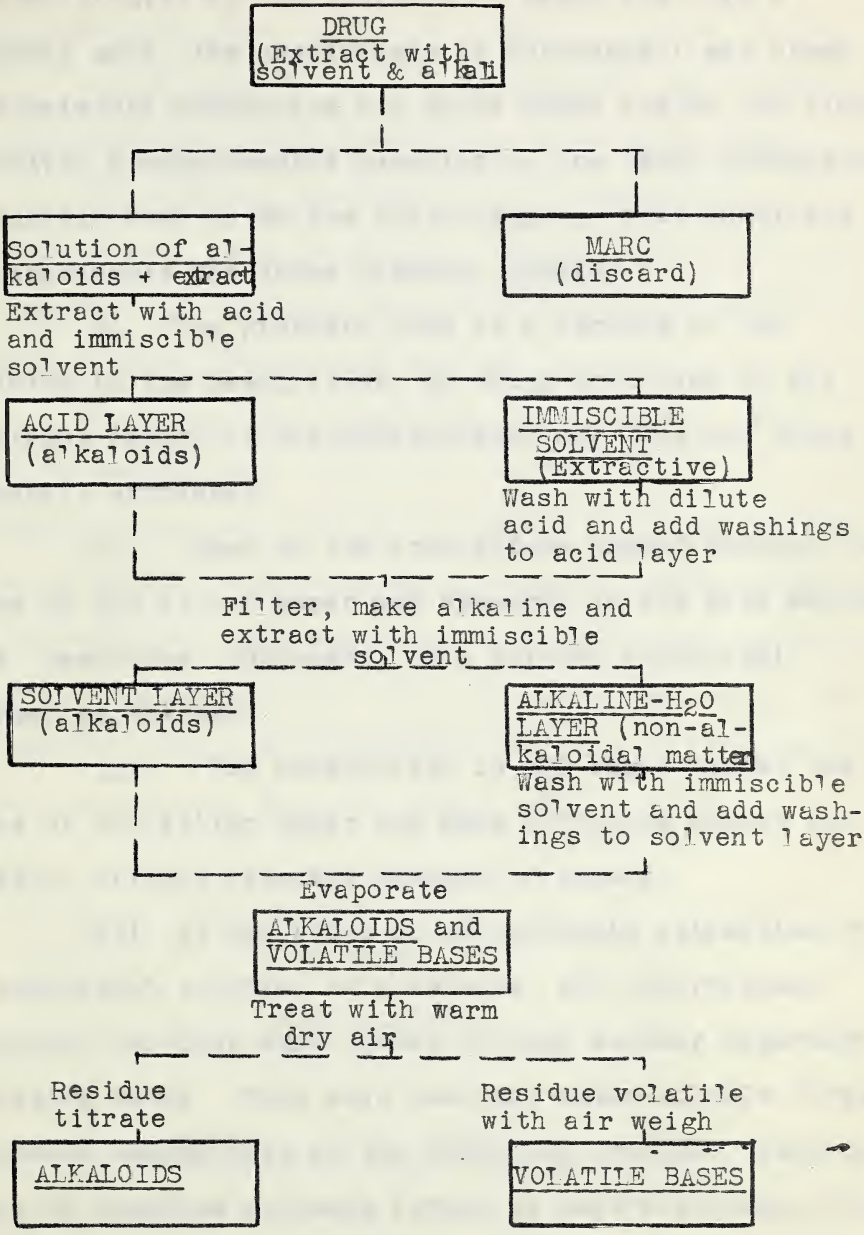

Plate III

In all cases, after the original ether solution was precipitated by the addition of water and 0.10 N sulphuric acid, the precipitate of chlorophyll and other non-alkaloidal extractive was quite bulky and on the first filtration a considerable quantity of the tarry precipitate was carried over on to the filter paper. This condition was undesirable for three reasons, namely,

i. The probable loss of a portion of the alkaloids in the precipitate, by being held back by the gelatinous nature of the precipitate, and thus not being thoroughly extracted.

ii. Much of the precipitate seeped through the meshes of the filter paper and appeared in the acid solution below, resulting, obviously, in a colored alkaloidal residue, at the end.

iii. The precipitate in all cases closed the meshes of the filter paper and made filtering almost impossible, without repeated changes of papers.

(b) At the point of chloroformic extraction of the ammoniacal solution of alkaloids, all chloroformic extraction portions were passed through another separator containing water. This held back any water-soluble foreign substances undesirable in the alkaloidal residue, such as traces of ammonium sulphate formed in neutralization. This procedure agreed with a paper appearing in 1915 (30) in which the writer suggested that a possible source of error

in all alkaloidal assays is the carrying down of ammonium salts, and recommended the washing of all final volatile solvent solutions.

With these two main modifications, the process was used throughout, as directed by DeKay and Jordan.

The process was used on all plants under investigation in order to obtain a comparative alkaloidal yield, after all volatile substances had been removed, and to compare these yields with the B.P. process.

(f) U.S.P.X. Method and Outline (29). This method is essentially similar to the B.P. 1932 method with the exception of the original technique, in that the drug is placed directly into the percolator and there macerated for one hour and then percolated. Since it was considered that the U.S.P.X. and B.P. 1932 processes were so similar that results should be comparable, the U.S.P.X. process was omitted in favor of the B.P. 1932.

V. EXPERIMENTAL

a. Test Garden

The test growing ground was a plot on the University of Alberta campus, adjoining the gardens. It was normal black soil, completely open to the sun, but well protected from wind by an efficient protective growth of trees and shrubs (see Plate IV, figs. 1 and 2). Fig. 1 shows the north-west corner, and fig. 2, the east side, of the test garden.

The experimental garden

Fig. 1

Fig. 2

Plate IV

b. The Seed

The seeds used in this investigation were obtained from various sources, in order to obtain offspring of plants grown under different climatic conditions. The seeds were obtained from the following sources:

1. The University of Minnesota, College of Pharmacy.

2. Mr. L. Tice, Westlock, Alberta.

3. Seed obtained from the previous summer's growth in this test garden.

In addition were used roots from the previous year's growth.

c. The Growth

All plants were grown in the same test garden and received the same amount of attention. The soil received no fertilizer. The plants during their growth received no special attention other than occasional watering and weeding.

The moisture needs of the plants provided quite a problem, as rainfall in central Alberta is not regular, and during some periods in the lifetime of Atropa Belladonna, rainfall was completely absent. The plants, as mentioned,

were watered when necessary, and as far as was possible without tests, were kept at optimum growing conditions.

1. Atropa Belladonna.

i. First year growth. The seed, obtained from University of Minnesota stock, was treated as suggested with concentrated sulphuric acid, for 45 seconds, repeatedly washed in a sieve with distilled water, and sown in the greenhouse on April 28th.

While the seedlings were still very small, they were transferred to the test garden, late in June. The plants were late in developing, but they withstood the severe frost (temperature 25°F.) which occurred on August 25th.

The plant produced several erect stems which did not produce a particularly dense foliage. At maximum growth, the plant was about three feet tall and produced characteristic flowers.

The leaves were collected on August 26th while at apparent maximum growth and when the plant was in flower. They were cut by hand, and avoiding as much as possible too much stem (the B.P. directs a maximum of 20% stem). Only clean, entire leaves were collected.

The freshly cut leaves were placed in large paper bags and without being pressed or unduly handled, transferred to the drying room in the basement, quickly spread in a thin, where possible single, layer on paper, on the floor. The room was maintained at an almost constant temperature of 50°.

Upon being thoroughly dried, the leaves were again gathered and ground in a Wiley power mill to a uniform #60 powder, weighed, the whole well mixed, bottled in amber, air-tight bottles and stored.

ii. Second year growth. All of the 1933 plants allowed to remain in the field over the winter were winter-killed. Two plants which had been potted and placed in the greenhouse the previous fall were set out in the field and grew to a height of about two and a half feet. In spite of the frost on August 25th and also numerous September frosts, these plants produced a few small seed-pods, two of which ripened. The leaves were all so completely frozen that they could not be collected.

2. Hyoscyamus niger.

i. First year growth. The seed for the 1934 growth of this plant was obtained from Mr. L. Tice of Westlock, Alberta, from plants grown by him in 1933.

The seeds were treated with concentrated sulphuric acid for one minute and 45 seconds before sowing.

Sowing was carried out on May 8th. The seedlings appeared about May 24th. The growth of the plant was fairly rapid.

The plant had a characteristic fetid odor and a heavy growth of the characteristically indented leaves was produced. The plant as a whole was low in stature with no upright stem.

The size of the leaves varied considerably. They were of a pale green color and while growing, had a soft and unpleasantly clammy surface. This stickiness was due to the soft hairs found near the veins, particularly on the under surface, which possess glandular heads secreting a resinous substance.

Two collections of leaves of the first year plants were made, the first on August 1st and the second on August 10th.

They were transferred to large paper bags and thence to the drying room where they were spread evenly and dried. They were then gathered, ground to a #60 powder, well mixed, bottled in well-closed containers and stored.

ii. Second year growth. The plants of the second year were grown from the roots of the first year growth, allowed to remain in the test garden over the winter, so that the conditions for growth for both first and second year plants may be said to be the same.

The roots of the plants sown in 1933 (which was L. Tice stock) left in place over the winter as mentioned above appeared to winter well.

The young shoots appeared above the ground about April 24th. The roots, soon after, were thinned out by transplanting, this work being carried out between April 27th and May 3rd.

The growth, as in the first year, was fairly rapid, but in this case the plant was considerably bulkier,

with a greater number of leaves and with a strong upright stem. At maximum growth, the characteristic flowers appeared in large numbers.

The plant, at the period of flowering, attained a growth of slightly more than three feet as may be seen in Plate V, fig. 3. The leaves had the characteristic indentations and were of a dull green color (Plate V, figs. 1, 2, 3).

On July 20th, the leaves were gathered, keeping in mind the limitations as to stems, directed by the B.P. The leaves were cut closely, transferred to large paper bags and spread in the drying room. When thoroughly dry, they were broken and passed through a Wiley mill to produce a #60 powder, thoroughly mixed, placed in well-closed containers and stored.

3. Datura stramonium.

The seed for this plant was obtained from the University of Minnesota stock. It was not treated with concentrated sulphuric acid, as was Atropa Belladonna, but was sown directly into the test garden on May 18th.

The growth of the plant was fairly rapid. The stems were round, very smooth and at maximum growth, woody.

The plant appeared to be more hardy than the others, and the growth seemed to bear out the statement of Koch (14) who stated that the plant required very little attention.

Hyoscyamus niger (second year)

Flowering plants

Fig. 1

Fig. 2

Fig. 3

Plate V

The plants, however, were cultivated as regularly as possible and watered occasionally. The growth of weeds was kept at a minimum. The plant produced quickly an abundant growth of light green foliage, with its deeply indented leaves and foul-smelling odor.

It grew to a height of about three feet, when the flowers appeared with their funnel shaped corollas. In rapid succession, the flowers gave way to the large 4-celled prickly walnut-shaped fruits.

The leaves were collected on August 16th, transferred to the drying room, dried, collected and ground to a #60 powder. The powder was well mixed, transferred to air-tight containers and stored.

Shortly after the leaves had been gathered, the life of the whole plant ended and the fruits began to open. The whole plant was then cut down, gathered, dried and the seeds threshed from the open capsules and a large yield of seed was obtained.

4. Datura Metel.

The seed was obtained from stock of the University of Minnesota, and sown on May 18th.

The growth of the plant was rapid and produced an abundance of leaves of a yellowish green color, which were irregular in shape. The odor of the plant, like Datura Stramonium, was unpleasant and characteristic. On the epidermis of the leaves occurred scattered simple as well

as small glandular hairs. At maximum growth, the plant produced white flowers.

No cultivation, other than occasional weeding and watering, was carried out on the plants.

The leaves were collected on August 16th in much the same manner as Datura Stramonium and the other solanaceous plants.

Here again, as little stem as possible was gathered and only the healthy clean leaves were used. The leaves were dried in the drying room at the constant temperature. When dry, they were gathered, ground to a #60 powder, well mixed, bottled in air-tight containers and stored.

d. Results of Determinations

1. Atropa Belladonna.

As will be noticed from the tabulated results, leaves of various years growth were used in the determination.

Since a considerable amount of 1931 and 1932 leaf was on hand, it was used for the first several determinations, until the technique in alkaloidal assays was perfected. The final results were taken from determinations carried out on leaf of 1933 and 1934 crops.

The first determination of 1933 leaf was that of moisture content. Then a determination of ash content was carried out, and finally alkaloidal assays by the DeKay and Jordan method and the B.P. 1932 method.

1. Atropa Belladonna (first year)

TABLE II

Moisture and ash content

Crop	Moisture %	Ash %	Remarks
1933	7.795	12.7155	
1933	8.298		
1934	6.954	11.8155	
1934	7.0923		

TABLE III

Alkaloidal content

Date	Crop	Method	Alkaloids %	Volatile bases %
May 12/34	1933	B.P.	0.2448	
"	"	"	0.2873	
May 15/34	"	"	0.4376	
"	"	"	0.4446	
Jan. 4/35	"	"	0.3688	
"	"	"	0.3617	
"	1934	"	0.3633*	
"	"	"	0.3798	
Jan.10/35	1933	"	0.3454+	
"	"	"	0.3400+	
"	1934	"	0.3410+	
"	"	"	0.3394+	
Jan.16/35	1933	DeK.& Jord.	0.3293**	
"	"	"	0.3275**	
"	1934	"	0.3314++	0.0045
"	"	"	0.2560++o	0.0035
Jan.18/35	"	"	0.3294**	
"	"	"	0.3306**	

* At ether-alcohol addition to leaf, became almost solid. 1934 leaf evidently drier than 1933.

\+ Residues not dried. Titrated by DeKay and Jordan method. Evidently lowers results.

** Volatile bases not removed and residue not divided.

++ Volatile bases removed but residues not divided.

o Partly lost.

** Volatile bases not removed and residue not divided.

2. Hyoscyamus niger (first year).

The stored powder representing Hyoscyamus niger (first year) was well mixed by turning it out on clean paper and alternately lifting the corners. It was then mixed with spatulas and replaced in the containers, from which samples were taken for the various determinations.

2. Hyoscyamus niger (first year)

TABLE IV

Moisture and ash content

Crop	Moisture %	Ash %	Remarks
1934	10.768	13.515	

TABLE V

Alkaloidal content

Date	Crop	Method	Alkaloids %	Volatile bases %
Feb.22/35	1934	B.P.	0.03413	
"	"	"	0.03430	
"	"	DeK.& Jord.	0.0664 *	0.0056
"	"	"	0.06312+	
"	"	"	0.06443*	0.0084
"	"	"	0.06434+	
Feb.26/35	"	B.P.	0.035015	
"	"	"	0.03378	
"	"	DeK.& Jord.	0.06263	0.0072
"	"	"	0.06189	0.0136

+ Volatile bases not removed.

* Volatile bases removed.

Hyoscyamus niger (second year)

TABLE VI

Moisture and ash content

Crop	Moisture %	Ash %	Remarks
1934	11.236	14.587	
"	10.940	14.590	

TABLE VII

Alkaloidal content

Date	Crop	Method	Alkaloids %	Volatile bases %
Feb. 1/35	1934	B.P.	0.02145+	
Feb. 5/35	"	"	0.04741	
"	"	"	0.04727	
Feb. 7/35	"	"	0.04110	
"	"	"	0.04147	
"	"	DeK.& Jord.	0.06034*	0.0047
"	"	"	0.06080*	0.0056
Feb. 7/35	"	"	0.07899**	
"	"	"	0.08012**	
Feb.14/35	"	B.P.	0.0438	
"	"	"	0.0433	
"	"	DeK.& Jord.	0.0763 *	0.0040
"	"	"	0.06052*	0.0068

+ Check lost.

* Volatile bases removed.

** Volatile bases not removed.

3. Datura Stramonium

TABLE VIII

Moisture and ash content

Crop	Moisture %	Ash %	Remarks
1932	4.755	13.8875	Water insoluble ash 49.3534%
1934	5.4800	13.852	

TABLE IX

Alkaloidal content

Date	Crop	Method	Alkaloids %	Volatile bases %
Feb.15/32	Penick	B.P.1914	0.3013	
"	"	"	0.2526	
Mar. 1/32	"	"	0.28562	
"	"	"	0.27612	
"	Local '31	"	0.18346*	
"	"	"	0.1822 *	
Nov.23/34	1934	B.P.1932	0.2545	
"	"	"	0.2519	
Nov.28/34	"	DeK.& Jord.	0.20115	0.0170
"	"	"	0.19805	0.0100
Dec.14/34	"	B.P.1932	0.20356	
"	"	"	0.1884 +	
Dec.18/34	"	DeK.& Jord.	0.2982	0.0125
"	"	"	0. - **	--
Dec.26/34	"	B.P.1932	0.170 ***	
"	"	"	0.154 ***	
"	"	"	0.22512	
"	"	"	0.22512	
"	"	DeK.& Jord.	0.2529	0.0120
"	"	"	0.2532	0.044

* First Alberta crop.

\+ Partly lost due to faulty separator.

** Sample B lost completely. A divided and part with volatile bases showed higher alkaloid (.302).2944 0.2982

*** Results low carried down considerable coloring matter. Titration difficult.

4. Datura Metel

TABLE X

Moisture and ash content

Crop	Moisture %	Ash %	Remarks
1934	7.104	14.99	
"	6.978	14.9112	

TABLE XI

Alkaloidal content

Date	Crop	Method	Alkaloids %	Volatile bases %
Feb. 7/35	1934	B.P.1932	0.2050	
"	"	"	0.2144	
Feb. 8/35	"	"	0.2138	
Feb.14/35	"	DeK.& Jord.	0.1947*	0.0088
"	"	"	0.2389+	
"	"	"	0.2234*	0.0076
"	"	"	0.2263+	
Feb.15/35	"	"	0.2270**	
"	"	"	0.2291**	

* Volatile bases removed.

\+ Volatile bases not removed.

** Residue not divided and volatile bases not removed.

VI. DISCUSSION

a. The Plant

In all cases, the plants under observation grew and matured with a growth similar to that described for the official B.P. plants found in southern Europe.

It was found that:

1. All plants were free of insect life and mildew.

2. The roots of Atropa Belladonna, at least, cannot resist the winter climate, in the field. They should be dug up and kept dormant in a warmer storage house and replanted in the spring for the second year's growth.

3. The roots of Hyoscyamus niger, left in the field, wintered well. Seeds planted for the first year on May 8th produced, from the wintered roots, shoots on the following April 24th. The plants produced by these wintered roots were hardy and had a healthy, rapid growth.

4. Datura Metel is the least suitable of the plants under observation, for Alberta. Only one plant produced seed pods, though it is an annual. All plants grown in the greenhouse, produced seed pods.

5. Atropa Belladonna must be started in the greenhouse, as the roots, as mentioned above, are killed in winter.

6. The roots of Hyoscyamus and Stramonium are hardy.

b. The Results

1. Moisture contents and ash values.

Ash contents were fairly consistent and all remained within the B.P. limits as shown. Various other values are given for ash values as follows:

Atropa Belladonna - Youngken* "Pharmacognoscy", maximum 20%.

Datura Stramonium - Youngken "Pharmacognoscy", maximum 20%.

In all cases the results obtained will be tabulated against the standards required by the B.P. and various other results, in a comparative manner.

TABLE XII

Moisture and ash values of solanaceous plants

Plant	Crop	Moisture %	Ash %	B.P. ash requirements
Atropa Belladonna	1933	8.047	12.7155	Maximum 15%
" "	1934	7.0232	11.8155	"
Hyoscyamus niger (1st year)	1934	10.7680	13.515	"
" " (2nd year)	1934	11.088	14.589	"
Datura Stramonium	1932	4.755	13.8875	Maximum 20%
" "	1934	5.480	13.852	"
Datura Metel	1934	7.041	14.9006	

* Published by P. Blakiston's Son & Co., Philadelphia. (1921).

Due to the slight variations in the moisture content of the drugs, and particularly to the urgent need in the Department of Pharmacy for an efficient drying oven, it is suggested that an oven with a maximum temperature range of 100° and capable of accommodating considerable fresh drug, be constructed.

The efficient drying of a drug is a very important factor in alkaloidal determinations as it has been suggested (Bentley "Text Book of Pharmaceutics"* page 260) that the alkaloids exist in the plant in the form of alkaloidal tanno-glucosides, which undergo partial decomposition when, on the death of the cell, the permeability of the plasma membrane changes and the acid cell sap enters and forms alkaloidal salts. This reaction is not complete, so that it is necessary to completely separate the alkaloids from the primary colloids. The decomposition in most cases takes place by means of the enzymes present, and these act best at a temperature of about 50°C.

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* Published by Bailliere, Tindall and Cox, London. (1933).

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* Published by Bell Telephone, Chelsea, Mass. 02150, U.S.A. (1933).

2. Alkaloidal contents.

TABLE XIII

Average alkaloidal contents of Solanaceae (B.P. method)

Plant	Crop	Method	Alkaloid %	B.P. requirement	Other references
Atropa Belladonna	1933	B.P.	0.3797	0.30	0.40%*
" "	1934	"	0.3059	0.30	0.40%**
Hyoscyamus niger	1934 (1st yr.)	"	0.0343	0.05	0.063%*
" "	1934 (2nd yr.)	"	0.04406	0.05	0.093%**
Datura Stramonium	1934	"	0.2321	0.25	0.22**
Datura Metel	1934	"	0.2111	--	0.40%* 0.50%**

* Henry "Plant Alkaloids", Published by J. and A. Churchill, London. (1924).
** Greenish "Materia Medica", Published by J. and A. Churchill, London. (1920).

TABLE XIV

Average alkaloidal contents of Solanaceae (DeKay and Jordan method)

Plant	Crop	Method	Alkaloid %	B.P. requirement	Other references
Atropa Belladonna	1933	DeK.& Jord.	0.3284	0.30	
" "	1934	"	0.3305	0.30	
Hyoscyamus niger (1st yr.)	1934	"	0.06342	0.050	
Hyoscyamus niger (2nd yr.)	1934	" (bases removed)	0.06449	0.050	0.0868% DeK.& Jord
Hyoscyamus niger (2nd yr.)	1934	" (bases not removed)	0.07956	0.050	0.1313% ibid.
Datura Stramonium	1934	DeK.& Jord.	0.2407	0.25	
" Metel	1934	" (bases removed)	0.2208		
" "	1934	" (bases not removed)	0.2268		

5. Artificial nonsense.

Table III

Average elasticity scores by [illegible] (S.D. omitted)

Items	Drop	Spring	Elasticity	S.E. Partial-ness	Other Difference
Strong Derivative 1924	0.5	"	0.0751	0.30	0.000*
" " 1924	"	"	0.0859	0.30	0.000**
Transylvania Liger 1924	"	"	0.1063	0.02	0.000*
" " 1924 (2nd yr.)	"	"	0.0999	0.02	0.000**
Datura Strandonius 1924	"	"	0.1227	0.02	0.000*
Datura Uebel 1924	"	"	0.2277	--	0.000*

* Henry Marsh Elasticity. Proceedings of the Royal Society (London). (1923).

** Greenfield "Quasi-Is Healing". Proceedings of the Royal Society London. (1923).

Table IV

Average elasticity coefficient of Strandonius (Drop) and Torsen Surface)

Item	Drop	Spring	Elasticity	S.E.	Other Difference
Strong Derivative 1924	0.5	Dec. 1924	0.0751	0.30	0.0*
" " 1924	"	"	0.0859	0.30	0.0*
Transylvania Liger 1924 (1st yr.)	"	"	0.0963	0.02	0.0*
Transylvania Liger 1924 (2nd yr.)	"	" (Loose Connected)	0.1063	0.02	0.0*
Transylvania Liger 1924 (3rd yr.)	"	" (Loose Not Connected)	0.0999	0.02	0.0*
Datura Strandonius 1924	Dec. 1924	Dec. 1924	0.1227	0.02	0.0*
" Uebel 1924	"	" (Loose Connected)	0.2277	--	0.0*
" " 1924	"	" (Loose Not Connected)	0.2277	--	0.0*

2. Alkaloidal contents.

A comparison of the results obtained on the plants by the two methods with the official requirements and results obtained by some other workers shows that by the B.P. 1932 method,

1. Atropa Belladonna is the only plant which produced alkaloid in excess of B.P. requirements, and very little lower than percentages tabulated from various reports in the text "Plant Alkaloids" by Henry (9) and "Materia Medica" by Greenish.

2. Hyoscyamus niger (both years) falls considerably below the B.P. requirement.

3. The second year Hyoscyamus growth is much richer in alkaloid than that of the first year.

4. Datura Stramonium falls very little below the B.P. standard, but is richer in alkaloid than the commercial samples which Greenish reports as containing an average of 0.22% alkaloids.

5. That Datura Metel (which did not produce seed) falls considerably below other standards as shown in the table.

The same plants when assayed by the DeKay and Jordan method show that:

1. Belladonna and Hyoscyamus both show an alkaloidal content considerably in excess of the B.P. requirements.

2. The second year's growth of Hyoscyamus is considerably richer in alkaloids than that of the first year.

3. Stramonium falls just below the B.P. requirements, and

4. Datura Metel, considerably below commercial samples.

Analysing then, the various tables, the following results are seen for the various plants.

TABLE XV

Alkaloidal contents of solanaceous plants when assayed by B.P. 1932 and DeKay and Jordan methods

	Crop	Atropa Belladonna	Hyoscyamus niger first year	Hyoscyamus niger second year	Datura Stramonium	Datura Metel
Growth	1934	Developed late	Abundant growth	Fairly rapid and heavy	Hardy and rapid	Flowering slow
Ash %	1934	11.815	13.515	14.589	13.852	14.9006
B.P. maximum		15.00	15.00	15.00	20.00	--
Moisture %	1934	7.0232	10.768	11.088	5.48	7.041
Alkaloids						
B.P. method	1933	0.3797	--	--	--	--
"	1934	0.3059	0.0343	0.04406	0.2321	0.2111
DeKay & Jordan	1933	0.3284	--	--	--	--
method	1934	0.3305	0.06342	0.0645	0.2407	0.2208
B.P. minimum		0.30	0.050	0.050	0.25	--

From this composite table will be seen that:

1. In all cases Atropa Belladonna contained alkaloid in excess of B.P. specifications.

2. Stramonium and Datura Metel are below the usual standards, as shown by both methods.

3. Hyoscyamus is below the requirements by the B.P. process and above by the DeKay and Jordan process.

4. Ash value in all plants is within B.P. limits.

3. Volatile bases.

In order to determine what effect, if any, the separation of volatile bases had on the remaining alkaloidal residue, several determinations were carried out with the following technique:

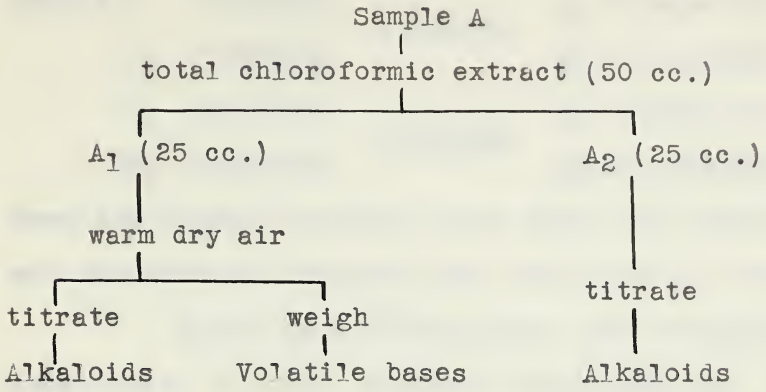
Two check samples A and B were weighed out as usual and the regular DeKay and Jordan extractions carried out to the point of the last chloroformic alkaloidal solution. At this point, instead of each solution being evaporated down with chloroform and acid, three times, the solution was transferred to a graduated 50 cc. cylinder and made up to 50 cc. with chloroform. Each sample was then accurately divided in half, each portion being transferred to a separate flask. Thus the samples became

$$A \begin{cases} A_1 & (25\ cc.) \\ A_2 & (25\ cc.) \end{cases}$$

$$B \begin{cases} B_1 & (25\ cc.) \\ B_2 & (25\ cc.) \end{cases}$$

each flask representing one-half of the original sample. The A_1 and B_1 samples were each freed of volatile bases,

while A_2 and B_2 were titrated directly. Diagrammatically this division and titration may be shown as



The DeKay and Jordan process appears to remove, by use of the warm air apparatus, all the volatile bases present. This can be seen by consulting Table VII, page 47, where on February 7/35, two determinations were carried out and directly titrated without extracting the volatile bases. The results obtained were:

0.07899%
0.08012%

Average 0.07956%

This result compares favorably with the other results in which the residues were originally treated with warm air, and gave an

Average 0.06449%

In the determinations in which the residues A and B were equally divided into A_1, A_2 and B_1, B_2 as outlined on page 56, it was found that the portions titrated without volatile bases removed were consistently higher as evidence

Table V (page 46) in which the four readings of two determinations are recorded.

Sample A_1	0.06312%	0.06476%	A_1 treated with air
A_2	0.06640%		A_2 not treated with air
B_1	0.06434%	0.06438%	B_1 treated with air
B_2	0.06443%		B_2 not treated with air

Here the average alkaloid when bases are removed is 0.06373% and the average alkaloid when bases are not removed is 0.06543%.

Since this investigation was interested essentially in total alkaloid determinations, the volatile bases and other residues brought down with the alkaloids were not identified. But in view of the various reports on substances brought down, it would prove an interesting investigation to enquire into the exact nature of all compounds extracted during a normal assay by the B.P. or mechanical methods, of a solanaceous plant. It may disclose many compounds brought down, which at present may be the cause of such various and differing results obtained, as pointed out by Sievers (24).

As mentioned, DeKay and Jordan (2) outlined their process with the object of isolating and weighing the volatile residue, which they definitely determined as di- and tri-methyl amines and pyridine. Goris and Larsonneau (8) isolated from Atropa Belladonna many volatile bases, an aliphatic amine, pyridine, N-methyl pyrolline, N-methyl pyrollidine, etc., all present in small quantities. It is possible, moreover, that many more compounds exist in the alkaloidal residue, besides

Table 2 (page 60) is where the good results of the
experiments are recorded.

Sample			
A_1	0.08752	0.08752	A_2 Treated with air
A_3	0.08698		A_4 Not treated with air
B_1	0.08624	0.08624	B_2 Treated with air
B_3	0.08644		B_4 Not treated with air

Here the average standard error seems low enough (< 0.00272)
and the average standard error seems low enough (< 0.00272).

Since this investigation was interested
[illegible] [illegible]. The
variable beans and other factors between them with the
standards were not [illegible]. But in view of the multi-
response in multivariate [illegible] case, it would prove an
interesting investigation to acquire data for about
[illegible] of all economic [illegible] series a number among
by the U.S. or mechanical methods, or a mathematical
[illegible]. It may [illegible] many [illegible] present time,
which at present may be the cause of many factors and
different results [illegible], as pointed out by [illegible].

As mentioned, under the [illegible] obtained
their success with the object of [illegible] the existing
[illegible] variable results, which [illegible] determined by
[illegible] and tri-dollar method and [illegible]. Some and
[illegible] (a) [illegible] these [illegible] [illegible] many
Variable bases, as [illegible] using, [illegible], D-methyl
[illegible]. 3-methyl [illegible], etc., [illegible] in
many [illegible]. It is [illegible], necessary. Many many
[illegible] [illegible] [illegible] [illegible]. Various

the above mentioned.

c. The Methods

Finally, the methods used may now be critically examined, with a view of determining their values or disadvantages.

1. The B.P. 1932 method.

There is no doubt that the B.P. 1932 assay methods for the solanaceous plants are tremendous improvements over the B.P. 1914 methods. The advantages of the former over the latter have been fully dealt with on pp. 23-28, the only factor in the procedure which may lead to error is the technique of titration.

All alkaloidal residues should be warmed slightly on a water-bath, after having had the addition of 0.02 N sulphuric acid.

All alkaloidal assays by the B.P. process carry down small amounts of colored residues, and if this be warmed while in contact with the acid, a greater degree of solution of alkaloids is affected. It should be then cooled to room temperature and titrated.

2. The DeKay and Jordan method (2).

This method in all cases save Atropa Belladonna 1933 crop (see Table XV, p. 55) gives a much higher alkaloidal content than does the B.P. process. This may be due to a number of factors.

i. Maceration. The extraction of the alkaloids from their natural state may, in this process, be more complete, since maceration is carried on over night. Also there is no chance of loss of drug, since there is no transfer from flask to percolator as in the B.P. method.

ii. Extraction. The extraction of freed alkaloids should, by this process as well as in the B.P. process, be complete, since the extractive is tested for absence of alkaloids in both cases.

iii. Titration. This process appears to have the better technique as applied to the titration of alkaloidal residues. There should be complete solution of alkaloid, and therefore complete titration of alkaloids present.

Though, as reported, various workers have had difficulty in obtaining consistent results by various methods, the above two methods gave, in this investigation, fairly consistent results.

VII. SUMMARY AND CONCLUSIONS

Observations and determinations were carried out on the following Alberta grown plants:

Atropa Belladonna

Hyoscyamus niger (first year)

Hyoscyamus niger (second year)

Datura Stramonium

Datura Metel

with a view of determining with what success these plants could be grown in central Alberta, from the viewpoint of both growth and alkaloidal content.

For the determination of the alkaloidal content of the various plants, two methods were used: (a) The B.P. 1932 method being an example of a cold percolation process; (b) The DeKay and Jordan method, being an example of a hot continuous extraction.

From the investigation the following conclusions were drawn.

1. The above plants can be successfully grown in central Alberta.

2. The roots of Atropa Belladonna cannot resist the winter climate.

3. The roots of Hyoscyamus niger can resist winter climate.

4. The moisture contents were fairly uniform.

5. The ash values were within the B.P. limits.

6. Atropa Belladonna produced alkaloid in excess of B.P. requirements.

7. Hyoscyamus niger (both years) falls below the alkaloidal requirements of the B.P. except by the DeKay and Jordan method.

8. Second year's growth of Hyoscyamus niger is richer in alkaloid than that of the first year.

9. Datura Stramonium falls just below the alkaloidal requirements of the B.P.

10. Datura Metel falls below the results given for commercial samples, and the growth is not particularly successful in Alberta.

11. By both methods used, and from results obtained, the solanaceous alkaloids are more stable than they are variously reported to be.

12. To avoid inaccurate results, plants containing volatile bases should be treated for their removal.

13. Using both methods on the same plants, the DeKay and Jordan method shows a much higher result, due probably, to more complete extraction.

VIII. ACKNOWLEDGMENTS

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